PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PU0242-PCT	FOR FURTHER ACTION See Form PCT/IPEA/416	
International application No. PCT/SE 2003/001127	International filing date *(day/month/year)* 26.06.2003	Priority date *(day/month/year)* 28.06.2002
International Patent Classification (IPC) or national classification and IPC C12N 15/10, C07H 1/06, C07H 1/08		
Applicant Amersham Biosciences AB et al		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 10 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☐ *(sent to the applicant and to the International Bureau)* a total of _____ sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) _____, containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:
   - ☒ Box No. I Basis of the report
   - ☐ Box No. II Priority
   - ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☐ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☒ Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
12.01.2004	03.08.2004
Name and mailing address of the IPEA/SE Patent- och registreringsverket Box 5055 S-102 42 STOCKHOLM Facsimile No. +46 8 667 72 88	Authorized officer Sara Nilsson/Els Telephone No. +46 8 782 25 00

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/SE 2003/001127

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ This report is based on a translation from the original language into the following language _____, which is the language of a translation furnished for the purposes of:

☐ international search (under Rules 12.3 and 23.1(b))
☐ publication of the international application (under Rule 12.4)
☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report):*

☒ the international application as originally filed/furnished

☐ the description:

pages _____ as originally filed/furnished
pages* _____ received by this Authority on _____
pages* _____ received by this Authority on _____

☐ the claims:

pages _____ as originally filed/furnished
pages* _____ as amended (together with any statement) under Article 19
pages* _____ received by this Authority on _____
pages* _____ received by this Authority on _____

☐ the drawings:

pages _____ as originally filed/furnished
pages* _____ received by this Authority on _____
pages* _____ received by this Authority on _____

☐ a sequence listing and/or any related table(s) – see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____
☐ the claims, Nos. _____
☐ the drawings, sheets/figs _____
☐ the sequence listing *(specify)*: _____
☐ any table(s) related to the sequence listing *(specify)*: _____

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

☐ the description, pages _____
☐ the claims, Nos. _____
☐ the drawings, sheets/figs _____
☐ the sequence listing *(specify)*: _____
☐ any table(s) related to the sequence listing *(specify)*: _____

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/SE 2003/001127

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application

☐ claims Nos. _____

because:

☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international preliminary examination *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. _____ are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for said claims Nos. 1-8, 10-17 all partially

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form
- ☐ has not been furnished
- ☐ does not comply with the standard

the computer readable form
- ☐ has not been furnished
- ☐ does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in the Annex C-*bis* of the Administrative Instructions.

☐ See Supplemental Box for further details.

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/SE 2003/001127

Box No. V **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-17	YES
	Claims		NO
Inventive step (IS)	Claims	16	YES
	Claims	1-15, 17	NO
Industrial applicability (IA)	Claims	1-17	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

The following documents are considered relevant:

D1) US4055469
D2) EP1031626 A1
D3) Izumrudov V.A. et al, "Controllable stability of DNA-containing polyelectrolyte complexes in water-salt solutions", Biopolymers (nucleic acid sciences), vol. 52, 94-108 (1999)
D4) Kabanov A. V. et al, "DNA interpolyelectrolyte complexes as a tool for efficient cell transformation, Biopolymers, vol. 31, 1437-1443 (1991)
D5) Zelikin A. N. And Izumrudov V. A. "Polyelectrolyte complexes formed by calf thymus DNA and aliphatic ionenes: unexpected change in stability upon variation of chain length of ionenes of different charge density", Macromol. Biosc. 2002, 2, 78-81
D6) EP0281390 A2
D7) US2002010145 A1
D8) Ramsden D. K. et al, "Flocculation of cellular material in complex fermentation medium with the flocculant poly(diallyldimethylammonium chloride)", Biotechnology techniques, vol. 12, no. 8, 1998
D9) US 5010183 A
D10) BIOSIS, accession number PREV19939610753 "Efficient separation of natural riobonucleotides by low-pressure anion-exchange chromatography"

D1 shows a method for precipitation of nucleic acids. The method can be used to selectively precipitate nucleic acids from a solution containing proteins. Cationic polymers, e.g. polymers containing quaternary amines are used in the method disclosed. The binding of the polymers to the nucleic acid,

.../...

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box V

the effectiveness of complex formation and precipitation, is not strongly influenced by the pH. To determine the quantity of polymer to be added the quantity of nucleic acids present in the extract can be determined. The effects of charge density and polymer size on the precipitation are investigated. The effect of solutions with different salt concentration is investigated. Nucleic acids are precipitated leaving other species in the solution. The precipitation is performed on cell lysates. See especially col. 4 lines 39-51, col. 5 lines 36-41, col. 7 lines 30-34, col. 8 lines 13-15 and col. 9 lines 24-32.

D2 shows the isolation of RNA and/or genomic DNA using cationic ammonium salts containing 1-24 repeating units. Nucleic acids are isolated from HeLa cells. The nucleic acid can be separated from the precipitation complex and isolated. See abstract, p. 4 line 18-p. 5 line 26 and p.38 claim 28.

In D3 the binding between DNA and e.g. poly(N',N'-dimethyldiallylammonium) chloride, ionene bromide or poly(N-alkyl-4-vinylpyridinium) is studied. The stability of the complexes at different salt concentrations is studied. By using a fluorescence spectroscopic assay, the formation of polyelectrolyte complex (PEC) is monitored and the charge ratio when the PEC is formed can be determined (the decrease in fluorescence seen when the charge ratio is about 1 or above 1, depending on the salt concentration). A method for monitoring the destruction of DNA-containing PECs in water-salt solutions is also disclosed. It is stated that PECs formed by polycations with quaternary amine groups are pH independent and the least tolerant to destruction of added salt. A mentioned application is delivery of DNA to cells. See p. 97 right col. Paragraph 3, p. 98 right col. and figure 1, p. 99 figure 3, p. 103 and p.105.

D4 relates to methods for increasing DNA hydrophobicity via inclusion into an interpolyelectrolyte complex with polycations. E.g. poly(N-ethyl-4-vinylpyridinium)bromide is used. Conditions under which self-assembly of DNA and polycation occurs, formation of an interpolyelectrolyte

.../...

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box V

complex (IPC), are established. It is shown that the formation of a soluble IPC occurs at a molar ratio of polycation repeating units and nucleic acid groups between 0 and 0,5. Parallel with the soluble IPC an insoluble complex with higher

polycation content is formed. A plasmid is incorporated into an IPC and transformed into cells. See p. 1439 left column.

D5 relates to polyelectrolyte complexes between DNA and aliphatic ionenes. It is stated that the degree of polymerisation and charge density of the ionenes control the stability of the complexes, which might be crucial for applications such as bioseparation. See p. 81 right col. paragraph 2.

D6 shows the use of polycationic solid supports in the purification of nucleic acids form solutions containing contaminants. The cations can be quaternary amines. The bound nucleic acids can be recovered from the support. See p. 6 lines 52-61, p. 7 line 85- p. 8 line 7, p. 17 example 15.

D7 shows a method for selective precipitation of DNA or plasmid DNA by the addition of a compaction agent such as spermidine or spermine. It is stated that the method can be performed on cell lysates. See abstract and fig. 1.

D8 shows the use of poly(diallyldimethylammonium chloride) for flocculation of cellular material. The charge density of the polymer used is 100.

D9 shows a method for purifying DNA or RNA from a mixture of biological materials, which comprises adding a cationic detergent to a mixture. The biological material mixture may be intact cells or cell lysates. The cationic detergent can be a quaternary amine cationic detergent such as an alkylbenzyldimethylammonium salt. The detergent is added in an amount sufficient to dissolve cells, solubilize any contaminating proteins and lipids in the mixture, and form insoluble hydrophobic complex between the nucleic acid and the detergent. The complex which comprises the RNA or DNA with the detergent is separated from the solubilized contaminants, and may be dissolved or dispersed in a polar organic solvent. Thereafter the DNA or RNA is recovered by the addition of a

.../...

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box V

salt, which promotes the dissociation of the complex. See col. 2 lines 48-52 and col. 3 lines 24-36.

D10 shows the use of anion exchangers containing quaternary ammonium functionalities for separating riobonucleotides.

The present application relates to the problem of selectively precipitating a nucleic acid from a solution containing other species while leaving said other species in the solution. This is achieved by using a polycationic precipitating agent being a highly charged linear polymer that comprises quaternary amino groups. The method allows precipitation within a broad window of pH values and salt concentrations and is not sensitive to addition of an excess of precipitating agent.

Document D1 is considered to represent the closest prior art.

The difference between the invention according to claim 1 and D1 is that the amount of precipitation agent (such an amount that the charge ratio [+]/[-] between polycationic precipitating agent and nucleic acid is ≥ about 0.5, preferably ≥ about 1) used in claim 1 is not is not specified in D1. In D1, the amount of polymer to be added is not determined on the basis of the charge ratio.

The expression "about 1" and "about 0,5" used in claim 1 makes the scope of the claim unclear (see. PCT Art. 6). It is not clear what "about 1" or "about 0,5" means. The optimal charge ratio for forming a specific complex depends upon the salt concentration, but the vague expressions "about 1" and "about 0,5" nevertheless make the scope of the claim unclear.

By adding precipitating agent in the amounts mentioned above, it seems that an insoluble precipitation complex is attained. The precipitation complex is attained within a broad window of pH values and salt concentrations and it is not sensitive to addition of an excess of precipitating agent.

Consequently, with the background of D1, the problem is to attain an insoluble precipitation complex and thus an efficient precipitation in relation to the aspects mentioned

.../...

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box V

above, when performing a precipitation of a nucleic acid using a highly charged linear polymer.

The skilled person faced with the problem mentioned above, finds the solution in D3, which discloses the theory of the stability of polyelectrolyte complexes depending on the charge ratio and the amount of polymer added. D3 shows that the

polymers poly(N',N'-dimethyldiallylammonium) chloride, ionene bromide and poly(N-alkyl-4-vinylpyridinium) bind to DNA and that the stability of the complexes can be controlled by varying e.g. the salt concentration. The skilled person would consider D3 since the document relate to the binding of DNA to polycations, as do D1. The technical application in D3 differs from the application in D1-D2 but the skilled person would combine the documents since they share the same theory. It is obvious to the skilled person that polyelectrolyte complexes can be used in bioseparation. Consequently, the invention according to claims 1-3, 5-12 and 17 is considered not to involve an inventive step given what is known form D1 in combination with D3. The addition of salt to dissolve or destruct the complex is investigated in D3. Consequently, the invention according to claims 11-15 is considered not to involve an inventive step given what is known from D1 in combination with D3.

The same argumentation as made above can be made starting with D2 or D8-D9 as the document representing the closest prior art. It can be mentioned that D2 and D9 show the recovery of the nucleic acids after separating the precipitate.

In present claim 1 the expression "which method comprises to selectively precipitate the desired nucleic acid, while leaving other species in solution" is used. By using this expression, the method is defined by reference to a result to be achieved by the method, not by technical features characterising how the method is performed. This way of defining the method leads to a lack of clarity (see PCT Art 6).

D1-D2 and D8-D9 show the precipitation of nucleic acids without the use of a strong base. Therefore, it is considered obvious to the skilled person that these methods can be used

.../...

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box V

to precipitate different kinds of nucleic acids. Consequently, the invention according to claim 4 is considered not to involve an inventive step.

Nothing is mentioned in either documents D1-D1 or D8-D9 about isolating more than one desired nucleic acid by continued addition of precipitating agent. Therefore, the invention according to claim 16 is not considered obvious to the skilled person in view of the sited documents.
Documents D4-D7 and D10 are considered to represent the general state of the art.

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

The expression "about 1" and "about 0,5" used in claim 1 makes the scope of the claim unclear (see. PCT Art. 6). It is not clear what "about 1" or "about 0,5" means. The optimal charge ratio for forming a specific complex depends upon the salt concentration, but the vague expressions "about 1" and "about 0,5" nevertheless make the scope of the claim unclear.

In present claim 1 the expression "which method comprises to selectively precipitate the desired nucleic acid, while leaving other species in solution" is used. By using this expression, the method is defined by reference to a result to be achieved by the method, not by technical features characterising how the method is performed. This way of defining the method leads to a lack of clarity (see PCT Art 6).